

## WHAT IS CLAIMED IS:

1. A method for amplifying a nucleotide sequence, characterized in that the method comprises:

(a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease;

(b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with the endonuclease at a site that contains the ribonucleotide; and

(c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement.

2. The method according to claim 1, wherein step (b) and step (c) are sequentially repeated.

3. A method for amplifying a nucleotide sequence using at least two primers, characterized in that the method comprises:

(a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease;

(b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with the endonuclease at a site that contains the ribonucleotide;

(c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (b);

(d) treating a released displaced strand obtained

in step (c) as a template with at least one primer that is different from that used in step (a) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer  
5 that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on  
10 the 3'-terminal side of the primer for cleavage with an endonuclease;

(e) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (d) with the endonuclease at a site that contains the ribonucleotide;  
15 and

(f) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which  
20 the primer-extended strand is cleaved obtained in step (e) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (e).

4. The method according to any one of claims 1  
25 to 3, wherein the respective steps are conducted

isothermally.

5. The method according to any one of claims 1 to 4, wherein one DNA polymerase having a strand displacement activity is used.

5 6. The method according to any one of claims 1 to 5, wherein the DNA polymerase is selected from the group consisting of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA  
10 polymerase lacking 5'→3' exonuclease from Bacillus caldotenax.

7. The method according to any one of claims 1 to 6, wherein the endonuclease is an endoribonuclease.

8. The method according to claim 7, wherein the  
15 endoribonuclease is RNase H.

9. The method according to any one of claims 1 to 8, wherein the chimeric oligonucleotide primer contains two or more successive ribonucleotide residues.

10. The method according to any one of claims 1  
20 to 9, wherein the chimeric oligonucleotide primer contains one or more modified ribonucleotide.

11. The method according to claim 10, wherein the chimeric oligonucleotide primer contains an ( $\alpha$ -S) ribonucleotide in which the oxygen atom bound to the  
25 phosphorous atom at the  $\alpha$ -position of the ribonucleotide is

replaced by a sulfur atom.

12. The method according to any one of claims 1 to 11, which is conducted in a buffer that contains a buffering component selected from the group consisting of  
5 Tricine, a phosphate and tris.

13. A method for amplifying a nucleotide sequence, characterized in that the method comprises:

(a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that  
10 cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a  
15 deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with the endonuclease; and

20 (b) incubating the reaction mixture for a sufficient time to generate a reaction product.

14. The method according to claim 13, wherein the reaction mixture is incubated isothermally.

15. The method according to claim 13 or 14,  
25 wherein the reaction mixture further contains a chimeric

oligonucleotide primer having a sequence substantially homologous to the nucleotide sequence of the nucleic acid as the template.

16. The method according to any one of claims 13  
5 to 15, wherein the DNA polymerase is selected from the group consisting of Klenow fragment of DNA polymerase I from *Escherichia coli*, Bst DNA polymerase lacking 5'→3' exonuclease from *Bacillus stearothermophilus* and Bca DNA polymerase lacking 5'→3' exonuclease from *Bacillus caldotenax*.  
10

17. The method according to any one of claims 13 to 16, wherein the endonuclease is an endoribonuclease.

18. The method according to claim 17, wherein the endoribonuclease is RNase H.

19. The method according to any one of claims 13  
15 to 18, wherein the chimeric oligonucleotide primer contains two or more successive ribonucleotide residues.

20. The method according to any one of claims 13 to 19, wherein the chimeric oligonucleotide primer contains  
20 one or more modified ribonucleotide.

21. The method according to claim 20, wherein the chimeric oligonucleotide primer contains an ( $\alpha$ -S) ribonucleotide in which the oxygen atom bound to the phosphorous atom at the  $\alpha$ -position of the ribonucleotide is  
25 replaced by a sulfur atom.

22. The method according to any one of claims 13 to 21, which is conducted in a buffer that contains a buffering component selected from the group consisting of Tricine, a phosphate and tris.

5 23. The method according any one of claims 1 to 22, wherein the nucleic acid as the template is a single-stranded DNA or a double-stranded DNA.

24. The method according to claim 23, which is conducted after converting a double-stranded DNA as the  
10 template into single-stranded DNAs.

25. The method according to claim 23 or 24, wherein the nucleic acid as the template is a cDNA obtained from an RNA by a reverse transcription reaction.

26. The method according to claim 25, which is  
15 conducted after synthesizing a cDNA by a reverse transcription reaction using an RNA as a template.

27. The method according to claim 26, wherein a primer selected from the group consisting of an oligo-dT primer, a random primer and a specific primer is used as a  
20 primer for the reverse transcription reaction.

28. The method according to claim 26 or 27, wherein a chimeric oligonucleotide primer is used as a primer for the reverse transcription reaction.

29. The method according to any one of claims 26  
25 to 28, wherein a DNA polymerase having a reverse

transcriptase activity is used as a reverse transcriptase.

30. The method according to any one of claims 26 to 29, wherein the reverse transcription reaction and the synthesis of the extended strand that is complementary to the template are conducted using one DNA polymerase having a reverse transcriptase activity and a strand displacement activity.

31. The method according to claim 28, wherein the DNA polymerase is Bst DNA polymerase lacking 5'→3' exonuclease from *Bacillus stearothermophilus* or Bca DNA polymerase lacking 5'→3' exonuclease from *Bacillus caldotenax*.

32. The method according to any one of claims 25 to 31, wherein the RNA as the template in the reverse transcription reaction is an RNA amplified by a nucleic acid amplification reaction.

33. The method according to claim 32, which is conducted after synthesizing an amplified RNA fragment by a nucleic acid amplification reaction using an RNA as a template.

34. The method according to claim 32 or 33, wherein the nucleic acid amplification reaction is selected from the group consisting of the transcription-based amplification system (TAS) method, the self-sustained sequence replication (3SR) method, the nucleic acid



sequence-based amplification (NASBA) method, the transcription-mediated amplification (TMA) method and the Q $\beta$  replicase method.

35. The method according to claim 23 or 24,  
5 wherein the nucleic acid as the template is a DNA obtained by a nucleic acid amplification reaction.

36. The method according to claim 35, which is conducted after synthesizing an amplified DNA fragment by a nucleic acid amplification reaction using an DNA as a  
10 template.

37. The method according to claim 36, wherein the nucleic acid amplification reaction is selected from the group consisting of the polymerase chain reaction (PCR) method, the ligase chain reaction (LCR) method and the  
15 strand displacement amplification (SDA) method.

38. The method according to any one of claims 32 to 37, wherein a random primer or a degenerate primer is used for the nucleic acid amplification reaction.

39. The method according to claim 38, wherein  
20 the random primer or the degenerate primer is a primer having a random sequence or a degenerate sequence at least at the 3'-terminus or on the 3'-terminal side.

40. A chimeric oligonucleotide primer used for the method for amplifying a nucleotide sequence according  
25 to any one of claims 1 to 39, which contains a

deoxyribonucleotide and a ribonucleotide, and has a structure in which the ribonucleotide is position at the 3'-terminus or on the 3'-terminal side of the primer.

41. The chimeric oligonucleotide primer  
5 according to claim 40, which contains two or more successive ribonucleotide residues.

42. The chimeric oligonucleotide primer according to claim 40 or 41, which contains one or more modified ribonucleotide.

10 43. The chimeric oligonucleotide primer according to claim 42, which contains an ( $\alpha$ -S) ribonucleotide in which the oxygen atom bound to the phosphorous atom at the  $\alpha$ -position of the ribonucleotide triphosphate is replaced by a sulfur atom as the modified  
15 ribonucleotide.

44. A DNA polymerase having a strand displacement activity used for the method for amplifying a nucleotide sequence according to any one of claims 1 to 39.

20 45. An endonuclease used for the method for amplifying a nucleotide sequence according to any one of claims 1 to 39.

25 46. A kit used for the method for amplifying a nucleotide sequence according to any one of claims 1 to 39, which is in a packaged form and contains instructions that direct the use of a DNA polymerase and an endonuclease in a

strand displacement reaction.

47. The kit according to claim 46, which contains a DNA polymerase and/or an endonuclease.

48. The kit according to claim 47, which is in a  
5 package form and contains:

(a) a DNA polymerase having a strand displacement activity;

(b) an endonuclease; and

(c) a buffer for a strand displacement reaction.

10 49. A kit used for the method for amplifying a nucleotide sequence according to any one of claims 1 to 39, which contains the DNA polymerase having the strand displacement activity according to claim 44 and/or the endonuclease according to claim 45.

15 50. The kit according to any one of claims 47 to 49, which contains a DNA polymerase selected from the group consisting of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA  
20 polymerase lacking 5'→3' exonuclease from Bacillus caldotenax as the DNA polymerase.

51. The kit according to any one claims 47 to 49, which contains RNase H as the endonuclease.

25 52. A method for detecting a target nucleic acid in a sample, characterized in that the method comprises:

(a) amplifying a target nucleic acid by the method for amplifying a nucleotide sequence according to claims 1 to 39; and

5 (b) detecting the target nucleic acid amplified in step (a).

10 53. The method according to claim 52, wherein the amplified target nucleic acid is detected using a ribonucleotide (RNA) probe labeled with two or more fluorescent substances positioned at a distance that results in a quenching state.

54. A DNA polymerase having a strand displacement activity used in the method for detecting a target nucleic acid according to claim 52 or 53.

15 55. An endonuclease used in the method for detecting a target nucleic acid according to claim 52 or 53.

20 56. A kit used for the method for detecting a target nucleic acid according to claim 52 or 53, which is in a packaged form and contains instructions that direct the use of a DNA polymerase and an endonuclease in a strand displacement reaction.

57. The kit according to claim 56, which contains a DNA polymerase and/or an endonuclease.

58. The kit according to claim 57, which is in a package form and contains:

25 (a) a DNA polymerase having a strand displacement

activity;

(b) an endonuclease; and

(c) a buffer for a strand displacement reaction.

59. The kit according to any one of claims 56 to  
5 58, which contains the DNA polymerase having the strand  
displacement activity according to claim 54 and/or the  
endonuclease according to claim 55.

60. The kit according to any one of claims 57 to  
59, which contains a DNA polymerase selected from the group  
10 consisting of Klenow fragment of DNA polymerase I from  
Escherichia coli, Bst DNA polymerase lacking 5'→3'  
exonuclease from Bacillus stearothermophilus and Bca DNA  
polymerase lacking 5'→3' exonuclease from Bacillus  
caldotenax as the DNA polymerase.

15 61. The kit according to any one claims 57 to 59,  
which contains RNase H as the endonuclease.

62. A method for producing a material having an  
immobilized nucleic acid in which the nucleic acid is  
arrayed in a predefined region, characterized in that the  
20 method comprises:

(a) amplifying a nucleic acid to be immobilized  
by the method for amplifying a nucleotide sequence  
according to any one of claims 1 to 39; and

(b) arraying and immobilizing the nucleic acid  
25 amplified in step (a) in a predefined region on a substrate.

63. The method according to claim 62, wherein a single-stranded nucleic acid substantially free of a complementary strand thereto is amplified, and arrayed and immobilized in the predefined region on the substrate.

5 64. A material having an immobilized nucleic acid in which the nucleic acid is arrayed in a predefined region produced by the method according to claim 62 or 63.

65. The material according to claim 64, wherein a single-stranded nucleic acid is arrayed and immobilized  
10 in the predefined region.

66. The material according to claim 65, in which a single-stranded nucleic acid substantially free of a complementary strand thereto is arrayed and immobilized in the predefined region.

15 67. A method for detecting a target nucleic acid in a sample, characterized in that the method comprises:

(a) preparing a nucleic acid sample suspected to contain a target nucleic acid from a sample;

(b) contacting the nucleic acid sample with the  
20 material according to any one of claims 64 to 66; and

(c) detecting the target nucleic acid in the nucleic acid sample that hybridizes with the nucleic acid on the material.

25 68. A method for producing a nucleic acid in large quantities, characterized in that the method

comprises:

(a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA  
5 polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on  
10 the 3'-terminal side of the primer for cleavage with an endonuclease;

(b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with the endonuclease at a site that contains the ribonucleotide;  
15 and

(c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which  
20 the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement.

69. A method for producing a nucleic acid in large quantities using at least two primers, characterized in that the method comprises:

25 (a) treating a nucleic acid as a template with at

least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a  
5 chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease;

10 (b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with the endonuclease at a site that contains the ribonucleotide;

(c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having  
15 a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended  
20 strand is reused in step (b);

(d) treating a released displaced strand obtained in step (c) as a template with at least one primer that is different from that used in step (a) and a DNA polymerase to synthesize a primer-extended strand that is  
25 complementary to the displaced strand, wherein the primer



that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a deoxyribonucleotide and a ribonucleotide, the  
5 ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease;

(e) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (d) with the  
10 endonuclease at a site that contains the ribonucleotide; and

(f) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the  
15 primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (e) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (e).

20 70. A method for producing a nucleic acid in large quantities, characterized in that the method comprises:

(a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide  
25 triphosphate, a DNA polymerase having a strand displacement

activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide  
5 sequence of the nucleic acid as the template and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with the endonuclease; and

10 (b) incubating the reaction mixture for a sufficient time to generate a reaction product.

71. A method for amplifying a nucleotide sequence, characterized in that the method comprises:

(a) amplifying a nucleic acid containing a  
15 sequence to be amplified by a nucleic acid amplification reaction to prepare a nucleic acid as a template;

(b) treating the nucleic acid as the template obtained in step (a) with at least one primer that is substantially complementary to the nucleotide sequence of  
20 the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the  
25 3'-terminus or on the 3'-terminal side of the primer for

cleavage with an endonuclease;

(c) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (b) with the endonuclease at a site that contains the ribonucleotide;  
5 and

(d) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which  
10 the primer-extended strand is cleaved obtained in step (c) to effect a strand displacement.

72. A method for amplifying a nucleotide sequence using at least two primers, characterized in that the method comprises:

15 (a) amplifying a nucleic acid containing a sequence to be amplified by a nucleic acid amplification reaction to prepare a nucleic acid as a template;

(b) treating the nucleic acid as the template obtained in step (a) with at least one primer that is  
20 substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a  
25 ribonucleotide, the ribonucleotide being positioned at the

3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease;

(c) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (b) with the endonuclease at a site that contains the ribonucleotide;

(d) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (c) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (c);

(e) treating a released displaced strand obtained in step (d) as a template with at least one primer that is different from that used in step (b) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer that is different from that used in step (b) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with an endonuclease;

(f) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (e) with the endonuclease at a site that contains the ribonucleotide; and

5 (g) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (f)  
10 to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (f).

73. A method for amplifying a nucleotide sequence, characterized in that the method comprises:

15 (a) amplifying a nucleic acid containing a sequence to be amplified by a nucleic acid amplification reaction to prepare a nucleic acid as a template;

(b) preparing a reaction mixture by mixing the nucleic acid as the template obtained in step (a), a  
20 deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary  
25 to the nucleotide sequence of the nucleic acid as the

template and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer for cleavage with the endonuclease; and

- 5 (c) incubating the reaction mixture for a sufficient time to generate a reaction product.

74. The method according to any one of claims 71 to 73, wherein the nucleic acid amplification reaction for preparing the nucleic acid as the template is selected from  
10 the group consisting of the TAS method, the 3SR method, the NASBA method, the TMA method, the Q $\beta$  replicase method, the PCR method, the LCR method and the SDA method.

75. The method according to claim 74, wherein a random primer or a degenerate primer is used for the  
15 nucleic acid amplification reaction.

76. The method according to claim 75, wherein the random primer or the degenerate primer is a primer having a random sequence or a degenerate sequence at least at the 3'-terminus or on the 3'-terminal side.

20 77. A method for amplifying a nucleotide sequence, characterized in that the method comprises:

(a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA  
25 polymerase to synthesize a primer-extended strand that is

complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with an endonuclease at a site that contains the ribonucleotide; and

(c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement.

78. A method for amplifying a nucleotide sequence using at least two primers, characterized in that the method comprises:

(a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the

ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with an endonuclease at a site that contains the ribonucleotide;

(c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (b);

(d) treating a released displaced strand obtained in step (c) as a template with at least one primer that is different from that used in step (a) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(e) cleaving the primer-extended strand of a



double-stranded nucleic acid obtained in step (d) with an endonuclease at a site that contains the ribonucleotide; and

(f) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (e) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (e).

79. A method for amplifying a nucleotide sequence, characterized in that the method comprises:

(a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; and

(b) incubating the reaction mixture for a

sufficient time to generate a reaction product.

80. A method for amplifying a nucleotide sequence, characterized in that the method comprises:

(a) treating a nucleic acid as a template with at  
5 least one primer that is substantially complementary to the  
nucleotide sequence of the nucleic acid and a DNA  
polymerase to synthesize a primer-extended strand that is  
complementary to the template, wherein the primer is a  
chimeric oligonucleotide primer containing a  
10 deoxyribonucleotide and a ribonucleotide, the  
ribonucleotide being positioned at the 3'-terminus or on  
the 3'-terminal side of the primer, wherein an endonuclease  
cleaves at a site that contains the ribonucleotide;

(b) cleaving the primer-extended strand of a  
15 double-stranded nucleic acid obtained in step (a) with the  
endonuclease at the site that contains the ribonucleotide;  
and

(c) extending a nucleotide sequence that is  
complementary to the template using a DNA polymerase having  
20 a strand displacement activity from the 3'-terminus of the  
primer portion of the double-stranded nucleic acid in which  
the primer-extended strand is cleaved obtained in step (b)  
to effect a strand displacement.

81. A method for amplifying a nucleotide  
25 sequence using at least two primers, characterized in that

the method comprises:

(a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA  
5 polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on  
10 the 3'-terminal side of the primer, wherein an endonuclease cleaves at a site that contains the ribonucleotide;

(b) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (a) with the endonuclease at the site that contains the ribonucleotide;

15 (c) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (b)  
20 to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (b);

(d) treating a released displaced strand obtained in step (c) as a template with at least one primer that is  
25 different from that used in step (a) and a DNA polymerase

to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer, wherein an endonuclease cleaves at a site that contains the ribonucleotide;

(e) cleaving the primer-extended strand of a double-stranded nucleic acid obtained in step (d) with the endonuclease at the site that contains the ribonucleotide; and

(f) extending a nucleotide sequence that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (e) to effect a strand displacement, wherein a double-stranded nucleic acid containing a regenerated primer-extended strand is reused in step (e).

82. A method for amplifying a nucleotide sequence, characterized in that the method comprises:

(a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide

triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease that cleaves an extended strand generated from the primer, wherein the primer is a chimeric oligonucleotide primer  
5 that is substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a deoxyribonucleotide and a ribonucleotide, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer, wherein the  
10 endonuclease cleaves at a site that contains the ribonucleotide; and

(b) incubating the reaction mixture for a sufficient time to generate a reaction product.

83. A method for determining a nucleotide  
15 sequence of a nucleic acid, characterized in that the method comprises amplifying a nucleotide sequence according to the method according to any one of claims 1 to 39 and 71 to 82.

84. A method for amplifying a nucleic acid,  
20 characterized in that the method comprises:

(a) preparing a reaction mixture by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an RNase H, wherein the  
25 primer is a chimeric oligonucleotide primer that is

substantially complementary to the nucleotide sequence of the nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer; and

(b) incubating the reaction mixture for a sufficient time to generate a reaction product.

85. The method according to claim 84, wherein the reaction mixture further contains a chimeric oligonucleotide primer having a sequence substantially homologous to the nucleotide sequence of the nucleic acid as the template.

86. A method for amplifying a nucleic acid, characterized in that the method comprises:

(a) treating a nucleic acid as a template with at least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template and synthesize a double-stranded nucleic acid, wherein the primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on

the 3'-terminal side of the primer;

(b) extending a nucleic acid that is complementary to the double-stranded nucleic acid as a template obtained in the previous step using a DNA  
5 polymerase having a strand displacement activity in the presence of an RNase H to effect a strand displacement and synthesize a displaced strand and a double-stranded nucleic acid; and

(c) reusing in step (b) the double-stranded  
10 nucleic acid obtained in step (b) as a template.

87. A method for amplifying a nucleic acid using at least two primers, characterized in that the method comprises:

(a) treating a nucleic acid as a template with at  
15 least one primer that is substantially complementary to the nucleotide sequence of the nucleic acid and a DNA polymerase to synthesize a primer-extended strand that is complementary to the template, wherein the primer is a chimeric oligonucleotide primer containing a ribonucleotide  
20 as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(b) extending a nucleic acid that is  
25 complementary to the double-stranded nucleic acid as a

template obtained in the previous step using a DNA polymerase having a strand displacement activity in the presence of an RNase H to effect a strand displacement and synthesize a displaced strand and a double-stranded nucleic acid;

(c) reusing in step (b) the double-stranded nucleic acid obtained in step (b) as a template;

(d) treating a displaced strand obtained in step (b) as a template with at least one primer that is different from that used in step (a) and a DNA polymerase to synthesize a primer-extended strand that is complementary to the displaced strand, wherein the primer that is different from that used in step (a) is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the displaced strand and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(e) extending a nucleic acid that is complementary to the double-stranded nucleic acid as a template obtained in the previous step using a DNA polymerase having a strand displacement activity in the presence of an RNase H to effect a strand displacement and synthesize a displaced strand and a double-stranded nucleic



acid; and

(f) reusing in step (e) the double-stranded nucleic acid obtained in step (e) as a template.

88. The method according to claim 86 or 87,  
5 wherein the DNA polymerase is at least one DNA polymerase having a strand displacement activity.

89. A method for amplifying a nucleic acid, characterized in that the method comprises:

10 (a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary  
15 to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisting of a  
20 deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid  
25 consisting of the primer-extended strands obtained in step

(a) with the endonuclease; and

(c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the  
5 respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the template and the primer-extended strand.

10 90. A method for amplifying a nucleic acid, characterized in that the method comprises:

(a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective  
15 strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being  
20 annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on  
25 the 3'-terminal side of the primer;

(b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease; and

5 (c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved  
10 obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the primer-extended strands being annealed each other.

91. A method for amplifying a nucleic acid, characterized in that the method comprises:

15 (a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to  
20 synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well  
25 as at least one selected from the group consisting of a

deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

(b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid consisting of the primer-extended strands obtained in step (a) with the endonuclease;

(c) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid in which the primer-extended strands are cleaved obtained in step (b) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the primer-extended strands being annealed each other and a double-stranded nucleic acid consisting of the templates being annealed each other to which the two primers in step (a) are annealed;

(d) extending nucleic acids that are complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-termini of the respective primer portions of the double-stranded nucleic acid to which the two primers are annealed obtained in step (c) to effect strand displacements and obtain a double-stranded nucleic acid consisting of the primer-extended

strands being annealed each other and a double-stranded nucleic acid consisting of the templates being annealed each other to which the two primers in step (a) are annealed; and

5 (e) reusing in step (d) the double-stranded nucleic acid to which the two primers are annealed obtained in step (d).

92. A method for amplifying a nucleic acid, characterized in that the method comprises:

10 (a) treating a double-stranded nucleic acid as a template with two primers that are substantially complementary to the nucleotide sequences of the respective strands of the double-stranded nucleic acid and a DNA polymerase having a strand displacement activity to  
15 synthesize primer-extended strands that are complementary to the template and obtain a double-stranded nucleic acid consisting of the synthesized primer-extended strands being annealed each other, wherein each primer is a chimeric oligonucleotide primer containing a ribonucleotide as well  
20 as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

25 (b) cleaving the sites that contain the ribonucleotide of the double-stranded nucleic acid

consisting of the primer-extended strands obtained in step  
(a) with the endonuclease;

(c) extending nucleic acids that are  
complementary to the template using a DNA polymerase having  
5 a strand displacement activity from the 3'-termini of the  
respective primer portions of the double-stranded nucleic  
acid in which the primer-extended strands are cleaved  
obtained in step (b) to effect strand displacements and  
obtain a double-stranded nucleic acid consisting of the  
10 primer-extended strands being annealed each other and a  
double-stranded nucleic acid consisting of the templates  
being annealed each other to which the two primers in step  
(a) are annealed;

(d) extending nucleic acids that are  
15 complementary to the template using a DNA polymerase having  
a strand displacement activity from the 3'-termini of the  
respective primer portions of the double-stranded nucleic  
acid to which the two primers are annealed obtained in step  
(c) to effect a strand displacement and obtain a double-  
20 stranded nucleic acid consisting of the template and the  
primer-extended strand;

(e) cleaving the sites that contain the  
ribonucleotide of the double-stranded nucleic acid  
consisting of the template and the primer-extended strand  
25 obtained in step (d) with the endonuclease; and

(f) extending a nucleic acid that is complementary to the template using a DNA polymerase having a strand displacement activity from the 3'-terminus of the primer portion of the double-stranded nucleic acid in which the primer-extended strand is cleaved obtained in step (e) to synthesize a displaced strand.

93. The method according to any one of claims 89 to 92, wherein the endonuclease is an endoribonuclease.

94. The method according to claim 93, wherein the endoribonuclease is an RNase H.

95. The method according to any one of claims 84 to 88 and 94, wherein the RNase H is selected from the group consisting of an RNase H from *Escherichia coli*, an RNase H from a bacterium of genus *Thermotoga*, an RNase H from a bacterium of genus *Thermus*, an RNase H from a bacterium of genus *Pyrococcus*, an RNase H from a bacterium of genus *Archaeoglobus* and an RNase H from a bacterium of genus *Bacillus*.

96. The method according to any one of claims 84 to 95, wherein the length of the region of the nucleic acid to be amplified is 200 bp or shorter.

97. The method according to any one of claims 84 to 96, wherein a chimeric oligonucleotide primer represented by general formula below is used:

General formula: 5'-dNa-Nb-dNc-3'

(a: an integer of 11 or more; b: an integer of 1 or more; c: 0 or an integer of 1 or more; dN: deoxyribonucleotide and/or nucleotide analog; N: unmodified ribonucleotide and/or modified ribonucleotide, wherein some of dNs in dNa may be replaced by Ns, and the nucleotide at the 3'-terminus may be modified such that extension from the 3'-terminus by the action of the DNA polymerase does not take place).

98. The method according to claim 97, wherein c is 0.

99. The method according to claim 97 or 98, wherein the nucleotide analog is deoxyriboinosine nucleotide or deoxyribouracil nucleotide, and the modified ribonucleotide is ( $\alpha$ -S) ribonucleotide.

100. The method according to any one of claims 97 to 99, wherein the DNA extension reaction is conducted at a DNA extension reaction temperature suitable for the chimeric oligonucleotide primer as defined in any one of claims 97 to 99.

101. The method according to any one of claims 84 to 100, which comprises annealing the nucleic acid as the template to the chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid in an annealing solution containing a substance that enhances the annealing of the nucleic acid



to the primer.

102. The method according to claim 101, wherein the annealing solution contains spermidine and/or propylenediamine.

5 103. The method according to claim 101 or 102, wherein the annealing is conducted by incubating the annealing solution containing the nucleic acid as the template and the chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of  
10 the nucleic acid at 90°C or above and then cooling the solution to a temperature at which the amplification reaction is conducted or below.

104. The method according to any one of claims 84 to 103, wherein the amplification reaction is conducted in  
15 a buffer containing a buffering component selected from the group consisting of Bicine and HEPES.

105. The method according to any one of claims 84 to 104, wherein the DNA polymerase having a strand displacement activity is selected from the group consisting  
20 of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3' exonuclease from Bacillus stearothermophilus and Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax.

106. The method according to any one of claims 84  
25 to 88 and 94 to 105, wherein the DNA polymerase having a

strand displacement activity is Bca DNA polymerase lacking 5'→3' exonuclease from *Bacillus caldotenax* and the RNase H is selected from the group consisting of an RNase H from *Escherichia coli*, an RNase H from a bacterium of genus *Pyrococcus* and an RNase H from a bacterium of genus *Archaeoglobus*.

107. The method according to claim 106, wherein the RNase H is type I RNase H from *Escherichia coli*, or type II RNase H from a bacterium of genus *Pyrococcus* or a bacterium of genus *Archaeoglobus*.

108. The method according to any one of claims 84 to 107, wherein a DNA polymerase having an endonuclease activity is used.

109. The method according to claims 108, wherein the DNA polymerase is Bca DNA polymerase lacking 5'→3' exonuclease from *Bacillus caldotenax* and the Bca DNA polymerase is used in the presence of a substance that allows the endonuclease activity of the Bca DNA polymerase to express.

110. The method according to claim 109, wherein the substance that allows the endonuclease activity of the DNA polymerase to express is a manganese ion.

111. The method according to any one of claims 84 to 110, wherein the amplification reaction is conducted in the presence of a substance that inhibits the reverse

transcription activity of the DNA polymerase.

112. The method according to claim 111, wherein the substance that inhibits the reverse transcription activity of the DNA polymerase is phosphonoformic acid.

5 113. The method according any one of claims 84 to 112, wherein the nucleic acid as the template is a single-stranded DNA or a double-stranded DNA.

114. The method according to claim 113, which is conducted after converting a double-stranded DNA as the  
10 template into single-stranded DNAs.

115. The method according to claim 113 or 114, wherein the nucleic acid as the template is a cDNA obtained by a reverse transcription reaction using an RNA as a template.

15 116. The method according to claim 115, which is conducted after synthesizing a cDNA by a reverse transcription reaction using an RNA as a template.

117. The method according to claim 115 or 116, wherein a DNA polymerase having a reverse transcriptase  
20 activity is used as a reverse transcriptase.

118. The method according to any one of claims 114 to 117, wherein the reverse transcription reaction and the synthesis of the extended strand that is complementary to the template are conducted using one DNA polymerase  
25 having both a reverse transcriptase activity and a strand

displacement activity.

119. The method according to claim 118, wherein the DNA polymerase is Bst DNA polymerase lacking 5'→3' exonuclease from *Bacillus stearothermophilus* or Bca DNA polymerase lacking 5'→3' exonuclease from *Bacillus caldotenax*.

120. The method according to any one of claims 84 to 119, wherein the nucleic acid amplification reaction is conducted under isothermal conditions.

121. The method according to any one of claims 84 to 120, wherein the nucleic acid amplification reaction is conducted in the presence of a deoxyribonucleotide triphosphate analog.

122. The method according to claim 121, wherein the deoxyribonucleotide triphosphate analog is deoxyuridine triphosphate or a derivative thereof.

123. A composition for amplifying a nucleic acid which contains:

(a) at least one primer that is substantially complementary to a nucleotide sequence of a nucleic acid as a template, wherein the primer is a chimeric oligonucleotide primer that contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on

the 3'-terminal side of the primer;

(b) an endonuclease; and

(c) a DNA polymerase having a strand displacement activity.

5           124. A composition for amplifying a nucleic acid which contains:

10           (a) at least two primers that are substantially complementary to nucleotide sequences of respective strands of a double-stranded nucleic acid as a template, wherein each primer is a chimeric oligonucleotide primer that contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer;

15           (b) an endonuclease; and

(c) a DNA polymerase having a strand displacement activity.

20           125. A composition for amplifying a nucleic acid obtained by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least one primer and an endonuclease, wherein the primer is a chimeric oligonucleotide primer that is substantially complementary to the nucleotide sequence of the nucleic acid as the  
25           template and contains a ribonucleotide as well as at least

one selected from the group consisting of a deoxyribonucleotide and a nucleotide analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer.

5           126. A composition for amplifying a nucleic acid obtained by mixing a nucleic acid as a template, a deoxyribonucleotide triphosphate, a DNA polymerase having a strand displacement activity, at least two primers and an endonuclease, wherein each primer is a chimeric  
10 oligonucleotide primer that is substantially complementary to the nucleotide sequence of each strand of the double-stranded nucleic acid as the template and contains a ribonucleotide as well as at least one selected from the group consisting of a deoxyribonucleotide and a nucleotide  
15 analog, the ribonucleotide being positioned at the 3'-terminus or on the 3'-terminal side of the primer.

127. The composition according to any one of claims 123 to 126, wherein the primer is a chimeric oligonucleotide primer represented by general formula  
20 below:

General formula: 5'-dNa-Nb-dNc-3'

(a: an integer of 11 or more; b: an integer of 1 or more; c: 0 or an integer of 1 or more; dN: deoxyribonucleotide and/or nucleotide analog; N: unmodified ribonucleotide  
25 and/or modified ribonucleotide, wherein some of dNs in dNa

may be replaced by Ns, and the nucleotide at the 3'-terminus may be modified such that extension from the 3'-terminus by the action of the DNA polymerase does not take place).

5           128. The composition according to claim 127, wherein c is 0.

10           129. The composition according to claim 127 or 128, wherein the nucleotide analog is deoxyriboinosine nucleotide or deoxyribouracil nucleotide, and the modified ribonucleotide is ( $\alpha$ -S) ribonucleotide.

15           130. The composition according to any one of claims 123 to 129, which contains a buffering component suitable for a nucleic acid amplification reaction.

20           131. The composition according to claim 130, which contains a buffering component selected from the group consisting of Bicine and HEPES.

25           132. The composition according to any one of claims 123 to 131, wherein a DNA polymerase selected from the group consisting of Klenow fragment of DNA polymerase I from *Escherichia coli*, Bst DNA polymerase lacking 5'→3' exonuclease from *Bacillus stearothermophilus* and Bca DNA polymerase lacking 5'→3' exonuclease from *Bacillus caldotenax* is used as the DNA polymerase having a strand displacement activity.

30           133. The composition according to any one of

claims 123 to 131, wherein the endonuclease is an endoribonuclease.

134. The composition according to claim 133, wherein the endoribonuclease is an RNase H.

5 135. The composition according to claim 134, wherein the RNase H is selected from the group consisting of an RNase H from *Escherichia coli*, an RNase H from a bacterium of genus *Thermotoga*, an RNase H from a bacterium of genus *Thermus*, an RNase H from a bacterium of genus  
10 *Pyrococcus*, an RNase H from a bacterium of genus *Archaeoglobus* and an RNase H from a bacterium of genus *Bacillus*.

136. The composition according to any one of claims 123 to 135, wherein the DNA polymerase having a  
15 strand displacement activity is Bca DNA polymerase lacking 5'→3' exonuclease from *Bacillus caldotenax* and the endonuclease is selected from the group consisting of an RNase H from *Escherichia coli*, an RNase H from a bacterium of genus *Pyrococcus* and an RNase H from a bacterium of  
20 genus *Archaeoglobus*.

137. The composition according to claim 136, wherein the RNase H is type I RNase H from *Escherichia coli*, or type II RNase H from a bacterium of genus *Pyrococcus* or a bacterium of genus *Archaeoglobus*.

25 138. The composition according to any one of



claims 123 to 137, wherein a DNA polymerase having an endonuclease activity is used.

139. The composition according to claims 138, wherein the DNA polymerase is Bca DNA polymerase lacking 5'→3' exonuclease from *Bacillus caldotenax*, the composition containing a substance that allows the endonuclease activity of the Bca DNA polymerase to express.

140. The composition according to claim 139, wherein the substance that allows the endonuclease activity of the DNA polymerase to express is a manganese ion.

141. The composition according to any one of claims 123 to 140, which contains a substance that inhibits the reverse transcription activity of the DNA polymerase.

142. The composition according to claim 141, wherein the substance that inhibits the reverse transcription activity of the DNA polymerase is phosphonoformic acid.

143. The composition according to any one of claims 123 to 142, which contains a deoxyribonucleotide triphosphate analog.

144. The composition according to claim 143, wherein the deoxyribonucleotide triphosphate analog is deoxyuridine triphosphate or a derivative thereof.

145. A composition for amplifying a nucleic acid used for the method for amplifying a nucleic acid according

to any one of claims 84 to 88, which contains:

(a) an RNase H; and

(b) a DNA polymerase having a strand displacement activity.

5           146. A composition for amplifying a nucleic acid used for the method for amplifying a nucleic acid according to any one of claims 89 to 92, which contains:

(a) an endonuclease; and

10           (b) a DNA polymerase having a strand displacement activity.

          147. The composition according to claim 146, wherein the endonuclease is an endoribonuclease.

          148. The composition according to claim 147, wherein the endoribonuclease is an RNase H.

15           149. The composition according to claim 145 or 148, wherein the RNase H is selected from the group consisting of an RNase H from *Escherichia coli*, an RNase H from a bacterium of genus *Thermotoga*, an RNase H from a bacterium of genus *Thermus*, an RNase H from a bacterium of  
20           genus *Pyrococcus*, an RNase H from a bacterium of genus *Archaeoglobus* and an RNase H from a bacterium of genus *Bacillus*.

          150. The composition according to any one of claims 145 to 149, which contains a buffering component  
25           suitable for a nucleic acid amplification reaction.

151. The composition according to claim 150, which contains a buffering component selected from the group consisting of Bicine and HEPES.

152. The composition according to any one of  
5 claims 145 to 151, wherein a DNA polymerase selected from the group consisting of Klenow fragment of DNA polymerase I from *Escherichia coli*, Bst DNA polymerase lacking 5'→3' exonuclease from *Bacillus stearothermophilus* and Bca DNA polymerase lacking 5'→3' exonuclease from *Bacillus*  
10 *caldotenax* is used as the DNA polymerase having a strand displacement activity.

153. The composition according to claim 145, wherein the DNA polymerase having a strand displacement activity is Bca DNA polymerase lacking 5'→3' exonuclease  
15 from *Bacillus caldotenax* and the RNase H is selected from the group consisting of an RNase H from *Escherichia coli*, an RNase H from a bacterium of genus *Pyrococcus* and a bacterium of genus *Archaeoglobus*.

154. The composition according to claim 146,  
20 wherein the DNA polymerase having a strand displacement activity is Bca DNA polymerase lacking 5'→3' exonuclease from *Bacillus caldotenax* and the endonuclease is selected from the group consisting of an RNase H from *Escherichia coli*, an RNase H from a bacterium of genus *Pyrococcus* and  
25 an RNase H from a bacterium of genus *Archaeoglobus*.

155. The composition according to any one of claims 145 to 154, wherein a DNA polymerase having an endonuclease activity is used.

5 156. The composition according to claims 155, wherein the DNA polymerase is Bca DNA polymerase lacking 5'→3' exonuclease from *Bacillus caldotenax*, the composition containing a substance that allows the endonuclease activity of the Bca DNA polymerase to express.

10 157. The composition according to claim 156, wherein the substance that allows the endonuclease activity of the DNA polymerase to express is a manganese ion.

158. The composition according to any one of claims 145 to 157, which contains a substance that inhibits the reverse transcription activity of the DNA polymerase.

15 159. The composition according to claim 158, wherein the substance that inhibits the reverse transcription activity of the DNA polymerase is phosphonoformic acid.

20 160. The composition according to any one of claims 145 to 159, which contains a deoxyribonucleotide triphosphate analog.

161. The composition according to claim 160, wherein the deoxyribonucleotide triphosphate analog is deoxyuridine triphosphate or a derivative thereof.

25 162. A kit for amplifying a nucleic acid used for

the method for amplifying a nucleic acid according to any one of claims 84 to 88, which contains:

- (a) an RNase H; and
- (b) a DNA polymerase having a strand displacement activity.

163. A kit for amplifying a nucleic acid used for the method for amplifying a nucleic acid according to any one of claims 89 to 92, which contains:

- (a) an endonuclease; and
- (b) a DNA polymerase having a strand displacement activity.

164. The kit according to claim 163, wherein the endonuclease is an endoribonuclease.

165. The kit according to claim 164, wherein the endoribonuclease is an RNase H.

166. The kit according to claim 162 or 165, wherein the RNase H is selected from the group consisting of an RNase H from *Escherichia coli*, an RNase H from a bacterium of genus *Thermotoga*, an RNase H from a bacterium of genus *Thermus*, an RNase H from a bacterium of genus *Pyrococcus*, an RNase H from a bacterium of genus *Archaeoglobus* and an RNase H from a bacterium of genus *Bacillus*.

167. The kit according to any one of claims 162 to 166, which contains a buffer suitable for a nucleic acid

amplification reaction.

168. The kit according to claim 167, which contains a buffer for nucleic acid amplification containing a buffering component selected from the group consisting of  
5 Bicine and HEPES.

169. The kit according to any one of claims 162 to 168, which contains an annealing solution containing a substance that enhances the annealing of the nucleic acid as the template to the primer that is substantially  
10 complementary to the nucleotide sequence of the nucleic acid.

170. The kit according to claim 169, wherein the annealing solution contains spermidine and/or propylenediamine.

171. The kit according to any one of claims 162 to 170, wherein a DNA polymerase selected from the group consisting of Klenow fragment of DNA polymerase I from Escherichia coli, Bst DNA polymerase lacking 5'→3'  
15 exonuclease from Bacillus stearothermophilus and Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus  
20 caldotenax is used as the DNA polymerase having a strand displacement activity.

172. The kit according to claim 162, wherein the DNA polymerase having a strand displacement activity is Bca  
25 DNA polymerase lacking 5'→3' exonuclease from Bacillus

caldotenax and the RNase H is selected from the group consisting of an RNase H from Escherichia coli, an RNase H from a bacterium of genus Pyrococcus and an RNase H from a bacterium of genus Archaeoglobus.

5           173. The kit according to claim 163, wherein the DNA polymerase having a strand displacement activity is Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax and the endonuclease is selected from the group consisting of an RNase H from Escherichia coli, an RNase H  
10           from a bacterium of genus Pyrococcus and an RNase H from a bacterium of genus Archaeoglobus.

          174. The kit according to claim 172 or 173, wherein the RNase H is type I RNase H from Escherichia coli, or type II RNase H from a bacterium of genus Pyrococcus or  
15           a bacterium of genus Archaeoglobus.

          175. The kit according to any one of claims 162 to 174, wherein a DNA polymerase having an endonuclease activity is used.

          176. The kit according to claims 175, wherein the  
20           DNA polymerase is Bca DNA polymerase lacking 5'→3' exonuclease from Bacillus caldotenax, the kit containing a substance that allows the endonuclease activity of the Bca DNA polymerase to express.

          177. The kit according to claim 176, wherein the  
25           substance that allows the endonuclease activity of the DNA

polymerase to express is a manganese ion.

178. The kit according to any one of claims 172 to 177, which contains a substance that inhibits the reverse transcription activity of the DNA polymerase.

5 179. The kit according to claim 178, wherein the substance that inhibits the reverse transcription activity of the DNA polymerase is phosphonoformic acid.

10 180. The kit according to any one of claims 162 to 179, which contains a deoxyribonucleotide triphosphate analog.

181. The kit according to claim 180, wherein the deoxyribonucleotide triphosphate analog is deoxyuridine triphosphate or a derivative thereof.

15 182. A kit for amplifying a nucleic acid used for the method for amplifying a nucleic acid according to any one of claims 84 to 88, which is in a packaged form and contains instructions that direct the use of a DNA polymerase having a strand displacement activity and an RNase H.

20 183. A kit for amplifying a nucleic acid used for the method for amplifying a nucleic acid according to any one of claims 89 to 92, which is in a packaged form and contains instructions that direct the use of a DNA polymerase having a strand displacement activity and an  
25 endonuclease.



184. A product of a reagent for amplifying a nucleic acid consisting of a packing material and a reagent for amplifying a nucleic acid enclosed in the packing material, wherein the reagent for amplifying a nucleic acid  
5 contains a DNA polymerase having a strand displacement activity and/or an RNase H, and description that the reagent for amplifying a nucleic acid can be used for nucleic acid amplification under isothermal conditions is indicated in a label stuck to the packaging material or  
10 instructions attached to the packaging material.

185. A product of a reagent for amplifying a nucleic acid consisting of a packing material and a reagent for amplifying a nucleic acid enclosed in the packing material, wherein the reagent for amplifying a nucleic acid  
15 contains a DNA polymerase having a strand displacement activity and/or an endonuclease, and description that the reagent for amplifying a nucleic acid can be used for nucleic acid amplification under isothermal conditions is indicated in a label stuck to the packaging material or  
20 instructions attached to the packaging material.

186. A method for detecting a target nucleic acid in a sample, characterized in that the method comprises:

(a) amplifying a nucleic acid by the method for amplifying a nucleic acid according to any one of claims 84  
25 to 122; and

(b) detecting a target nucleic acid amplified in step (a).

187. The method according to claim 186, which comprises detecting the amplified nucleic acid using a probe for detection.

188. The method according to claim 187, wherein the probe for detection is a probe that has been labeled with a labeling substance.

189. The method according to claim 188, wherein the probe is an RNA probe labeled with two or more fluorescent substances positioned at a distance that results in a quenching state.

190. A chimeric oligonucleotide primer used for the method for detecting a target nucleic acid according to any one of claims 186 to 189.

191. The chimeric oligonucleotide primer according to claim 190 which is represented by general formula below:

General formula: 5'-dNa-Nb-dNc-3'

(a: an integer of 11 or more; b: an integer of 1 or more; c: 0 or an integer of 1 or more; dN: deoxyribonucleotide and/or nucleotide analog; N: unmodified ribonucleotide and/or modified ribonucleotide, wherein some of dNs in dNa may be replaced by Ns, and the nucleotide at the 3'-terminus may be modified such that extension from the 3'-

terminus by the action of the DNA polymerase does not take place).

192. The chimeric oligonucleotide primer according to claim 191, wherein c is 0.

5 193. The chimeric oligonucleotide primer according to claim 191 or 192, wherein the nucleotide analog is deoxyribinosine nucleotide or deoxyribouracil nucleotide, and the modified ribonucleotide is ( $\alpha$ -S) ribonucleotide.

10 194. The chimeric oligonucleotide primer according to any one of claims 190 to 193, which is a chimeric oligonucleotide primer for detecting a pathogenic microorganism or a disease-related gene.

15 195. The chimeric oligonucleotide primer according to claim 194, wherein the pathogenic microorganism is enterohemorrhagic *Escherichia coli*, *Clostridium botulinum*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Chlamydia*, papilloma virus, hepatitis C virus or a viroid.

20 196. A chimeric oligonucleotide primer for detecting enterohemorrhagic *Escherichia coli* having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 31 to 34, 47, 48, 51-53, 64-72, 84, 85, 113, 114, 130 and 131.

25 197. A chimeric oligonucleotide primer for

detecting a viroid having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 59, 60, 119, 120, 122 and 123.

5 198. A chimeric oligonucleotide primer for detecting *Clostridium botulinum* having a nucleotide sequence represented by SEQ ID NO: 116 or 117.

199. A chimeric oligonucleotide primer for detecting papilloma virus having a nucleotide sequence represented by SEQ ID NO: 96 or 97.

10 200. A chimeric oligonucleotide primer for detecting hepatitis C virus having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 101, 102, 138, 139, 200, 201, 205 and 206.

15 201. A chimeric oligonucleotide primer for detecting *Staphylococcus aureus* having a nucleotide sequence represented by SEQ ID NO: 136 or 137.

20 202. A chimeric oligonucleotide primer for detecting *Mycobacterium tuberculosis* having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 155, 156, 159 to 162, 194 and 195.

203. A chimeric oligonucleotide primer for detecting *Chlamydia* having a nucleotide sequence selected from the group consisting of SEQ ID NOS: 157, 158, 203 and 204.

25 204. A kit for amplifying a nucleic acid used for

the method for amplifying a nucleic acid according to any one of claims 84 to 122, which contains the chimeric oligonucleotide primer according to any one of claims 190 to 203.

5           205. A kit for detecting a target nucleic acid used for the method for detecting a target nucleic acid according to any one of claims 186 to 189, which contains the chimeric oligonucleotide primer according to any one of claims 190 to 203.

10           206. A probe used in the method according to any one of claims 186 to 189.

          207. A probe which hybridizes to the nucleic acid amplified by the method according to any one of claims 84 to 122.

15           208. A probe which hybridizes to a region amplified using the chimeric oligonucleotide primer according to any one of claims 196 to 203.

          209. The probe according to any one of claims 206 to 208, which has been labeled with a labeling substance.

20           210. The probe according to claim 209, which is an RNA probe labeled with two or more fluorescent substances positioned at a distance that results in a quenching state

          211. A kit used in the method according to any  
25 one of claims 186 to 189, which contains the probe

according to any one of claims 206 to 210.

212. A method for amplifying a nucleic acid,  
which comprises using a DNA polymerase having a strand  
displacement activity to effect a template switching  
5 reaction.

213. The method according to claim 212, wherein  
the DNA polymerase having a strand displacement activity is  
selected from the group consisting of Klenow fragment of  
DNA polymerase I from Escherichia coli, Bst DNA polymerase  
10 lacking 5'→3' exonuclease from Bacillus stearothermophilus  
and Bca DNA polymerase lacking 5'→3' exonuclease from  
Bacillus caldotenax.

214. A method for producing a material having an  
immobilized nucleic acid in which the nucleic acid is  
15 arrayed in a predefined region, characterized in that the  
method comprises:

(a) amplifying a nucleic acid to be immobilized  
by the method for amplifying a nucleic acid according to  
any one of claims 84 to 122; and

20 (b) arraying and immobilizing the nucleic acid  
amplified in step (a) in a predefined region.

215. A material having an immobilized nucleic  
acid in which the nucleic acid is arrayed in a predefined  
region produced by the method according to claim 214.

25 216. A method for producing a nucleic acid in

large quantities, characterized in that the method comprises:

(a) amplifying a nucleic acid by the method for amplifying a nucleic acid according to any one of claims 84 to 122; and

(b) collecting the nucleic acid amplified in step (a).

217. A method for amplifying a nucleic acid, characterized in that the method comprises:

(a) duplicating a DNA or an RNA containing a sequence to be amplified to prepare a nucleic acid as a template; and

(b) amplifying the nucleic acid as the template obtained in step (a) by the method for amplifying a nucleic acid according to any one of claims 84 to 122.

218. A method for determining a nucleotide sequence of a nucleic acid, characterized in that the method comprises amplifying a nucleic acid according to the method according to any one of claims 84 to 122, 216 and 217.

219. A method for preparing a single-stranded nucleic acid, the method comprising a step of generating a single-stranded nucleic acid using the method according to any one of claims 84 to 122.

220. The method according to claim 219, wherein

at least two primers at different concentrations are used.